



LoopDeetect Food Safety

Gene amplification detection method

Protocols

-

Proteinase K Centrifugation

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Contents

Protocols with PK and centrifugation	4
SWA_09 – Surface swabbing / PK / Centrifugation / PFS+ manual purification	5
SWA_10 – Surface swabbing / PK / LoopiX® lysis / Centrifugation / PurifiX®8 purification	8
SWA_11 – Surface swabbing / PK / Heating block / Centrifugation / PurifiX®8 purification	11
SWA_12 – Surface swabbing / PK / Centrifugation / PurifiX® 10 purification	14
MIL_09 – Enriched medium / PK / Centrifugation / PFS+ manual purification	17
MIL_10 - Enriched medium / PK / LoopiX® lysis / Centrifugation / PurifiX®8 purification	20
MIL_11 - Enriched medium / PK / Heating block / Centrifugation / PurifiX®8 purification	23
MIL_12 - Enriched medium / PK / Centrifugation / PurifiX®10 purification	26
COL_09 – Colony isolated from agar plate / PK / Centrifugation / PFS+ manual purification	29
COL_10 – Colony isolated from agar plate / PK / LoopiX® lysis / Centrifugation / PurifiX®8 purification	32
COL_11 – Colony isolated from agar plate / PK / Heating block / Centrifugation / PurifiX®8 purification	35
COL_12 – Colony isolated from agar plate / PK / Centrifugation / PurifiX®10 purification	38
NEG_01 – Negative control	41
POS_01 – Positive control	41
INH_01 – Inhibition control	42
Document history / Version tracking	45

Important information

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To achieve optimal results, it is essential to follow the operating procedures and recommendations for use strictly. The protocol to be applied depends, in particular, on the type of matrices, the user and the equipment used.

Before use, the user must have read and understood all the user manuals for the equipment and products used as part of the protocol. The user manuals are available on the following page: [Loop Dee Science – Download](#)

The user manuals specific to the **LoopDeetect AOAC** kits are also available on the following page: [Loop Dee Science - Download](#)

Protocols with PK and centrifugation

	PFS+ purification	PurifiX 8 purification	PurifiX 10 purification
Surface swabbing	<u>SWA 09</u>	<u>SWA 10</u> <u>SWA 11</u>	<u>SWA 12</u>
Food or environmental sample in a medium	<u>MIL 09</u>	<u>MIL 10</u> <u>MIL 11</u>	<u>MIL 12</u>
Colony isolated from agar plate	<u>COL 09</u>	<u>COL 10</u> <u>COL 11</u>	<u>COL 12</u>
Negative control	<u>NEG 01</u>		
Positive control	<u>POS 01</u>		
Inhibition control	<u>INH 01</u>		

SWA_09 – Surface swabbing / PK / Centrifugation / PFS+ manual purification

Sampling

- Moisten the swab with buffer **S1**.
- Take the surface sample using the swab.
- Immerse the swab head in tube **L1** and release the sample contents by rotating and moving the swab back and forth for **15 seconds**, then press the swab against the side of the tube to extract the liquid.

Lysis

- Using a pipette fitted with a new tip, transfer **25 µL** of **liquid PK** into tube **L1** and mix thoroughly by pipetting back and forth.
- Close tube **L1** and place it in the LoopiX®.
- Using the control interface, start a '**PK and amplification**' analysis. The indicator light flashes red throughout the lysis process.
- At the end of lysis, the indicator light flashes blue. Remove tube **L1** from the LoopiX®.
- Using a pipette fitted with a tip or a 6 mL transfer pipette, aspirate the entire contents of tube **L1**.

Centrifugation

- Whilst lysis is taking place, prepare the separation column:
 - Take a separation column and detach it from the **C1** collection tube.
 - Remove the cap from the column.
 - Reinsert the column into the collection tube.
- Transfer the entire sample from tube **L1** into the separation column.
- Close the column and place it in the centrifuge. Balance the centrifuge.
- Start centrifugation at **2500 xg** (maximum 6000 xg) for **2 minutes**.

NB: The time/speed combination may be set specifically according to the centrifuge model; please specify the relevant data below:

Speed	Duration

- After centrifugation, remove the separation column from its collection tube **C1**. Discard the column.
- Using a pipette fitted with a new tip, or a new 6 mL bulb pipette, collect all of the filtrate from tube **C1**.

If a pellet has formed at the bottom of tube **C1**, ensure that only the supernatant is collected.

Purification with PFS+ kit

- Transfer the lysate (liquid remaining after the lysis step) to tube **B2** using a 6 mL pipette or a pipette fitted with a tip.
- Homogenise by aspirating and dispensing 15 times, ensuring the beads are evenly distributed without clumping.

- Wait 1 minute, then place tube **B2** on the magnetic stand to capture the beads.
- Discard the supernatant without touching the beads, leaving **B2** on the stand.
- Add buffer **W3** and homogenise again.
- Place tube **B2** on the magnetic stand to capture the beads.
- Discard the supernatant without touching the beads; tube **B2** remains on the stand.
- Add buffer **W4** and repeat the steps for homogenisation, bead capture and removal of the supernatant.
- Add buffer **W5** and repeat the same steps of homogenisation, bead capture and supernatant removal.
- Dry the bottom of the tube with a Lemoine swab or a pipette fitted with a **new tip** (nothing should remain at the bottom of the tube), then discard the swab or tip.
- Leave to air dry for 5 minutes with tube **B2** open.
- Using a 3.5 mL pipette or a pipette fitted with a new tip, add buffer **E6** and homogenise. Wait 1 minute.
- Place tube **B2** on the magnetic stand to capture the beads.
- Using a pipette fitted with a **new tip**, withdraw **25 µL** from tube **B2** without touching the beads.

Analysis

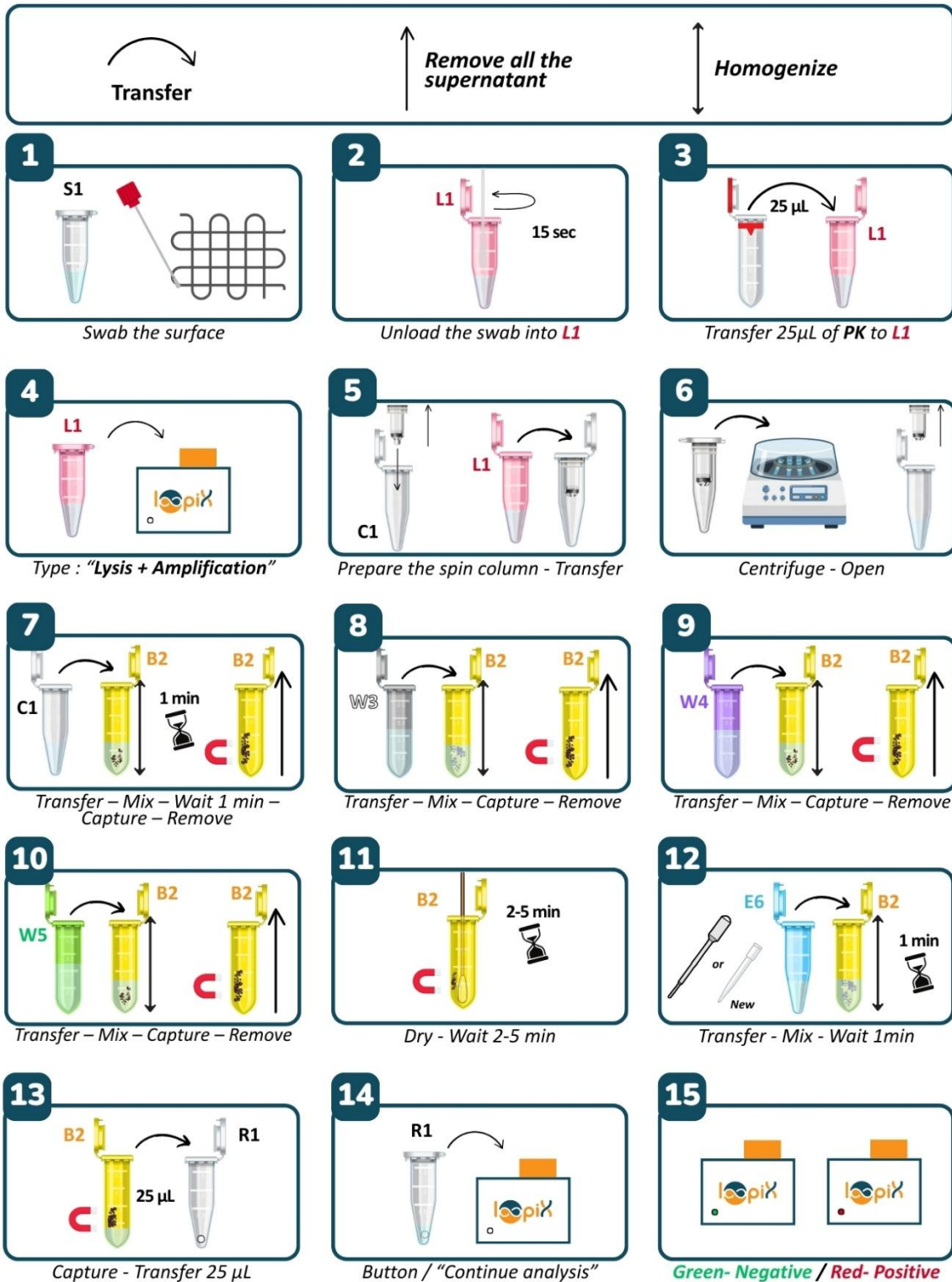
- Transfer the **25 µL** to reaction tube **R1**.
- Seal tube **R1** by pressing down with your finger until you hear a 'click' indicating that it is securely closed, then place it in the LoopiX®.
- Start the analysis by pressing the button on the front of the LoopiX® or via the control interface.
- At the end of the analysis, read the result directly from the status LED and on the control interface:
 - **Red LED:** positive sample
 - **Green LED:** negative sample

End of analysis

- Discard the **R1** reaction tube and **NEVER open it**.
- Discard all consumables used for the analysis.
- Using an alcohol-based solution and an absorbent cloth, clean the work area, tools (pipettes, stands, etc.) and equipment (LoopiX®, PurifiX®, etc.)
- Perform a 30-minute UV sterilisation cycle for equipment featuring this function.

SWA_09

Surface swabbing /PK LoopiX® Lysis/ Centrifugation/ PFS+ manual purification



SWA_10 – Surface swabbing / PK / LoopiX® lysis / Centrifugation / PurifiX® 8 purification

Sampling

- Moisten the swab with buffer **S1**.
- Take the surface sample using the swab.
- Immerse the swab head in tube **L1** and release the sample contents by rotating and moving the swab back and forth for **15 seconds**, then press the swab against the side of the tube to extract the liquid.

Lysis

- Using a pipette fitted with a new tip, transfer **25 µL** of **liquid PK** into tube **L1** and mix thoroughly by pipetting back and forth.
- Close tube **L1** and place it in the LoopiX®.
- Using the control interface, start a '**PK and amplification**' analysis. The indicator light flashes red throughout the lysis process.
- At the end of lysis, the indicator light flashes blue. Remove tube **L1** from the LoopiX®.
- Using a pipette fitted with a tip or a 6 mL transfer pipette, aspirate the entire contents of tube **L1**.

Centrifugation

- Whilst lysis is taking place, prepare the separation column:
 - Take a separation column and detach it from the **C1** collection tube.
 - Remove the cap from the column.
 - Reinsert the column into the collection tube.
- Transfer the entire sample from tube **L1** into the separation column.
- Close the column and place it in the centrifuge. Balance the centrifuge.
- Start centrifugation at **2500 xg** (maximum 6000 xg) for **2 minutes**.

NB: The time/speed combination may be set specifically according to the centrifuge model; please specify the relevant data below:

Speed	Duration

- After centrifugation, remove the separation column from its collection tube **C1**. Discard the column.
- Using a pipette fitted with a new tip, or a new 6 mL bulb pipette, collect all of the filtrate from tube **C1**.

If a pellet has formed at the bottom of tube **C1**, ensure that only the supernatant is collected.

PurifiX® 8 purification

- Transfer the lysate (liquid remaining after the lysis step) to **well 1** of the PurifiX® 8 purification strip using a 6 mL pipette or a pipette fitted with a tip.
- Place the purification strip onto the holder in the PurifiX® 8.

- Insert the comb(s) into the device, ensuring they are pushed all the way in.
- Close the instrument lid and start the run using the control interface.
- At the end of the purification, discard the comb(s) and remove the purification strip.
- Using a pipette fitted with a **new tip**, aspirate **25 µL** from **well 6** of the strip and proceed with the analysis.

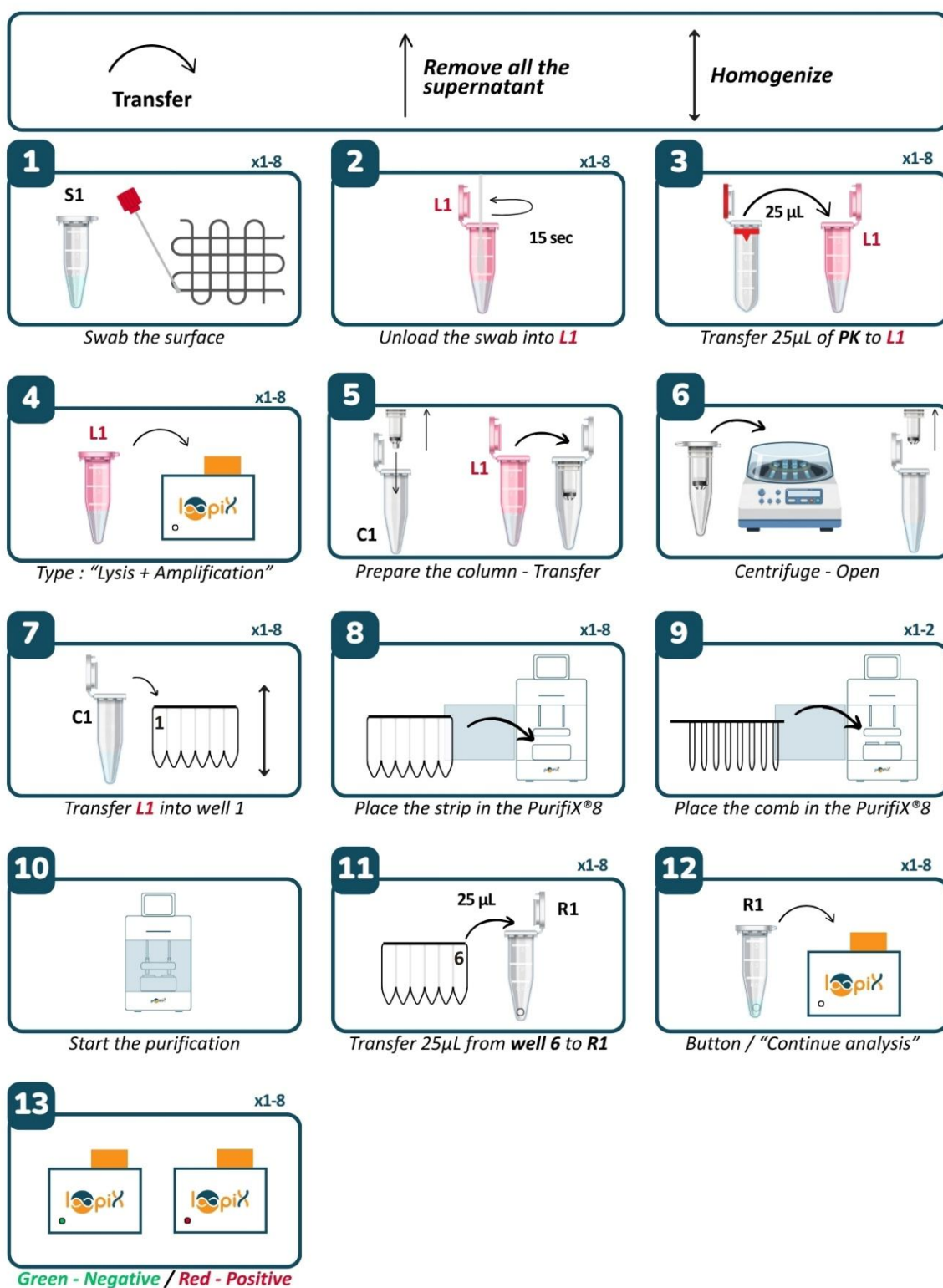
Analysis

- Transfer the **25 µL** to reaction tube **R1**.
- Seal tube **R1** by pressing down with your finger until you hear a 'click' indicating that it is securely closed, then place it in the LoopiX®.
- Start the analysis by pressing the button on the front of the LoopiX® or via the control interface.
- At the end of the analysis, read the result directly from the status LED and on the control interface:
 - **Red LED:** positive sample
 - **Green LED:** negative sample

End of analysis

- Discard the **R1** reaction tube and **NEVER open it**.
- Discard all consumables used for the analysis.
- Using an alcohol-based solution and an absorbent cloth, clean the work area, tools (pipettes, stands, etc.) and equipment (LoopiX®, PurifiX®, etc.)
- Perform a 30-minute UV sterilisation cycle for equipment featuring this function.

SWA_10 Surface swabbing / PK/ Lysis LoopiX® / Centrifugation / PurifiX®8



SWA_11 – Surface swabbing / PK / Heating block / Centrifugation / PurifiX®8 purification

Sampling

- Moisten the swab with buffer **S1**.
- Take the surface sample using the swab.
- Immerse the swab head in tube **L1** and release the sample contents by rotating and moving the swab back and forth for **15 seconds**, then press the swab against the side of the tube to extract the liquid.

Lysis

- Using a pipette fitted with a new tip, transfer **25 µL** of **liquid PK** into tube **L1** and mix by pipetting back and forth.
- Seal tube **L1** and place it in the heating block preheated to **65°C**.
- Set a timer for **10 minutes**.
- Once lysis is complete, remove tube **L1** from the heating block and wait 2 minutes for it to cool.
- Using a pipette fitted with a tip or a 6 mL transfer pipette, aspirate the entire contents of tube **L1**.

Centrifugation

- Whilst lysis is taking place, prepare the separation column:
 - Take a separation column and detach it from the **C1** collection tube.
 - Remove the cap from the column.
 - Reinsert the column into the collection tube.
- Transfer the entire sample from tube **L1** into the separation column.
- Close the column and place it in the centrifuge. Balance the centrifuge.
- Start centrifugation at **2500 xg** (maximum 6000 xg) for **2 minutes**.

NB: The time/speed combination may be set specifically according to the centrifuge model; please specify the relevant data below:

Speed	Duration

- After centrifugation, remove the separation column from its collection tube **C1**. Discard the column.
- Using a pipette fitted with a new tip, or a new 6 mL bulb pipette, collect all of the filtrate from tube **C1**.

If a pellet has formed at the bottom of tube **C1**, ensure that only the supernatant is collected.

PurifiX® 8 purification

- Transfer the lysate (liquid remaining after the lysis step) to **well 1** of the PurifiX® 8 purification strip using a 6 mL pipette or a pipette fitted with a tip.
- Place the purification strip onto the holder in the PurifiX® 8.
- Insert the comb(s) into the device, ensuring they are pushed all the way in.

- Close the instrument lid and start the run using the control interface.
- At the end of the purification, discard the comb(s) and remove the purification strip.
- Using a pipette fitted with a **new tip**, aspirate **25 µL** from **well 6** of the strip and proceed with the analysis.

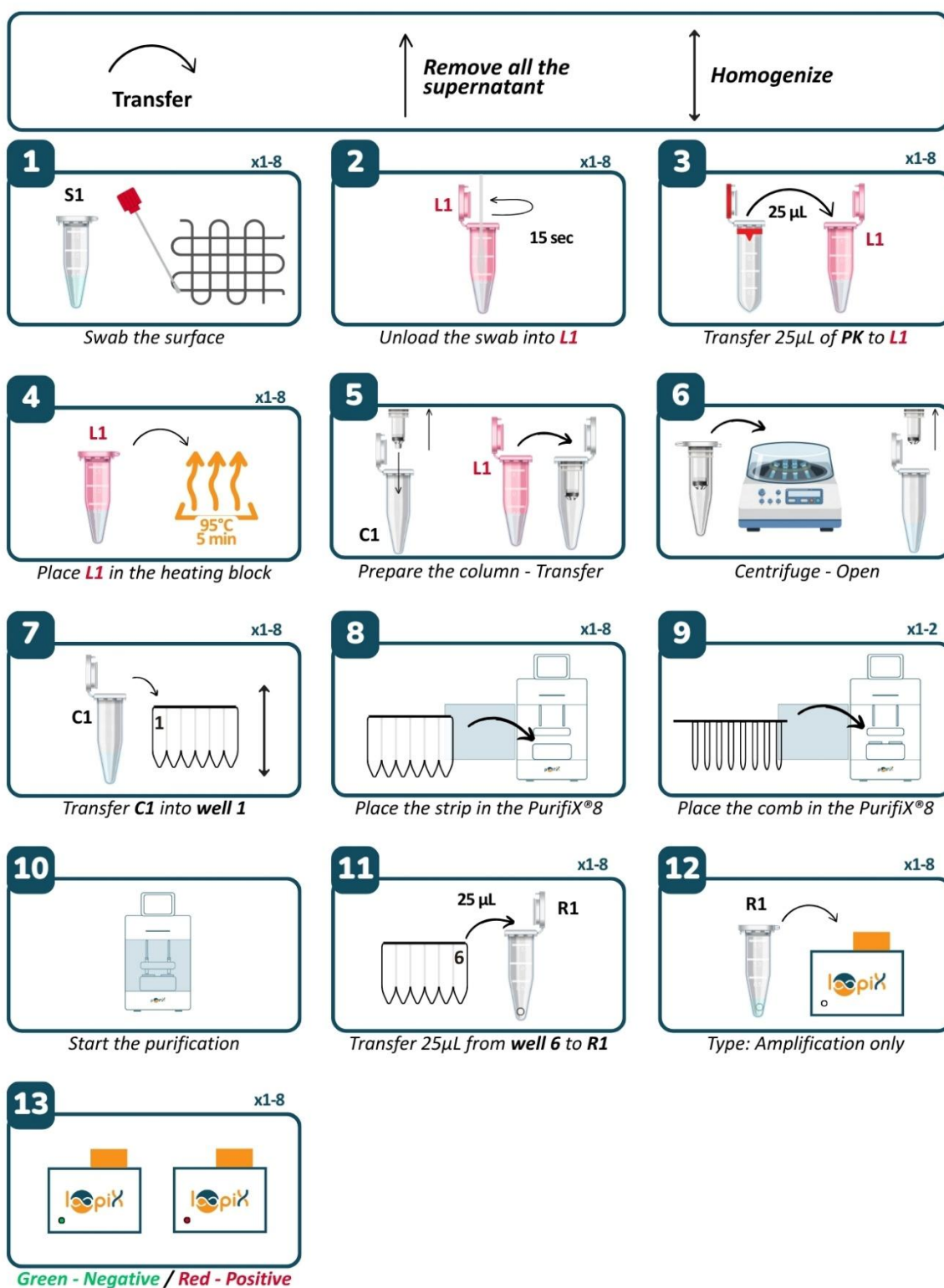
Analysis

- Transfer the **25 µL** to reaction tube **R1**.
- Seal tube **R1** by pressing down with your finger until you hear a 'click' indicating that it is securely closed, then place it in the LoopiX®.
- Start the analysis by pressing the button on the front of the LoopiX® or via the control interface.
- At the end of the analysis, read the result directly from the status LED and on the control interface:
 - **Red LED:** positive sample
 - **Green LED:** negative sample

End of analysis

- Discard the **R1** reaction tube and **NEVER open it**.
- Discard all consumables used for the analysis.
- Using an alcohol-based solution and an absorbent cloth, clean the work area, tools (pipettes, stands, etc.) and equipment (LoopiX®, PurifiX®, etc.)
- Perform a 30-minute UV sterilisation cycle for equipment featuring this function.

SWA_11 Surface swabbing / PK/ Heating block / Centrifugation / PurifiX®8



SWA_12 – Surface swabbing / PK / Centrifugation / PurifiX® 10 purification

Sampling

- Moisten the swab with buffer **S1**.
- Take the surface sample using the swab.
- Immerse the swab head in tube **L1** and release the sample contents by rotating and moving the swab back and forth for **15 seconds**, then press the swab against the side of the tube to extract the liquid.

Lysis

- Using a pipette fitted with a new tip, transfer **25 µL** of **liquid PK** into tube **L1** and mix by pipetting back and forth.
- Seal tube **L1** and place it in the heating block preheated to **65°C**.
- Set a timer for **10 minutes**.
- Once lysis is complete, remove tube **L1** from the heating block and wait 2 minutes for it to cool.
- Using a pipette fitted with a tip or a 6 mL transfer pipette, aspirate the entire contents of tube **L1**.

Centrifugation

- Whilst lysis is taking place, prepare the separation column:
 - Take a separation column and detach it from the **C1** collection tube.
 - Remove the cap from the column.
 - Reinsert the column into the collection tube.
- Transfer the entire sample from tube **L1** into the separation column.
- Close the column and place it in the centrifuge. Balance the centrifuge.
- Start centrifugation at **2500 xg** (maximum 6000 xg) for **2 minutes**.

NB: The time/speed combination may be set specifically according to the centrifuge model; please specify the relevant data below:

Speed	Duration

- After centrifugation, remove the separation column from its collection tube **C1**. Discard the column.
- Using a pipette fitted with a new tip, or a new 6 mL bulb pipette, collect all of the filtrate from tube **C1**.

If a pellet has formed at the bottom of tube **C1, ensure that only the supernatant is collected.**

PurifiX® 10 purification

- Transfer the lysate (liquid remaining after the lysis phase) to **well 1** of the PurifiX® 10 purification strip using a 6 mL pipette or a pipette fitted with a tip.
- Place the purification strip into the PurifiX® 10.
- Place the comb into the rail, ensuring it is pushed all the way in.

- Close the instrument lid and start the run using the control interface.
- Once purification is complete, discard the comb and remove the purification strip.
- Using a pipette fitted with a **new tip**, withdraw **25 µL** from **well 7** of the strip and proceed with the analysis.

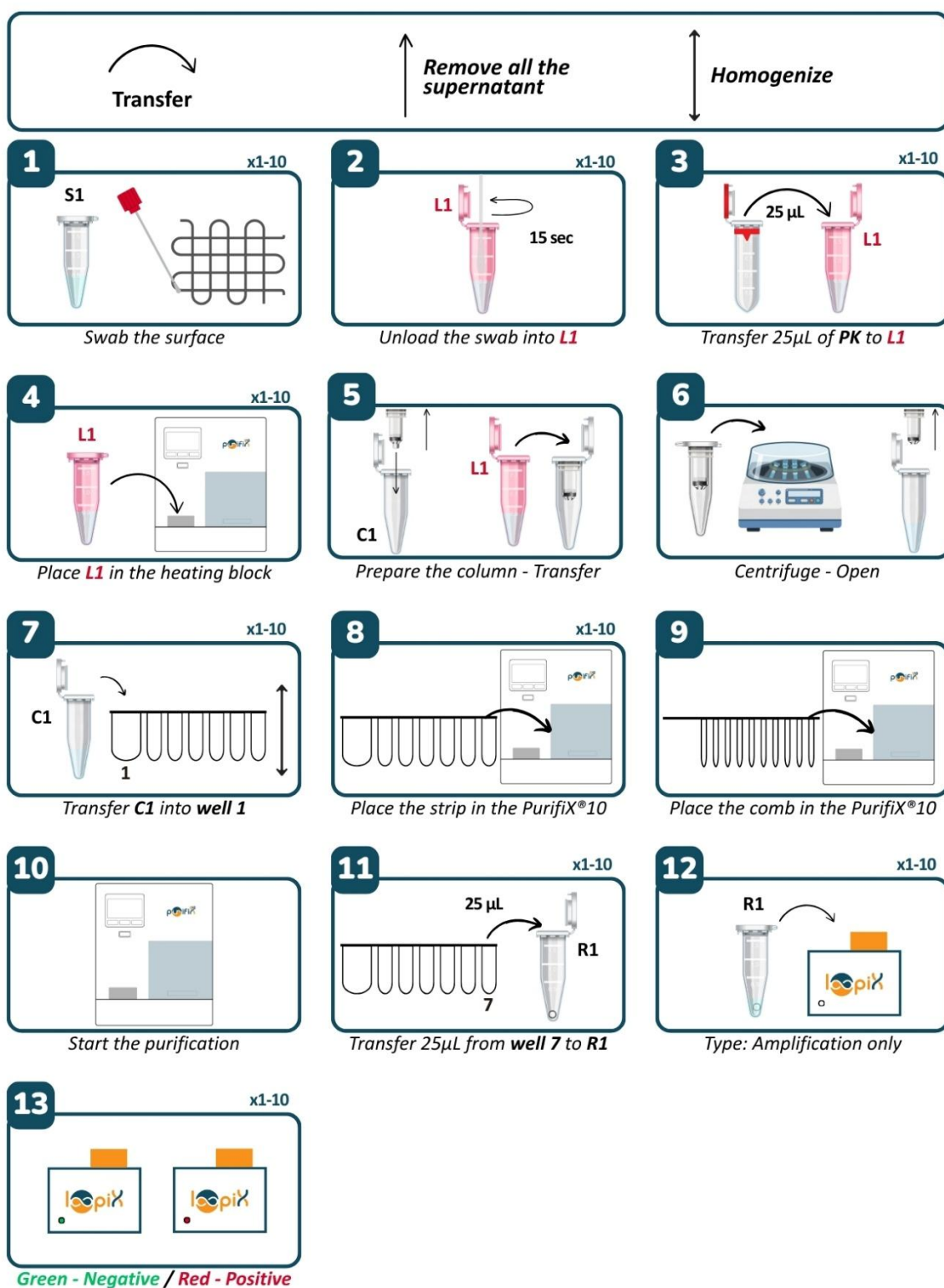
Analysis

- Transfer the **25 µL** to reaction tube **R1**.
- Seal tube **R1** by pressing down with your finger until you hear a 'click' indicating that it is securely closed, then place it in the LoopiX®.
- Start the analysis by pressing the button on the front of the LoopiX® or via the control interface.
- At the end of the analysis, read the result directly from the status LED and on the control interface:
 - **Red LED:** positive sample
 - **Green LED:** negative sample

End of analysis

- Discard the **R1** reaction tube and **NEVER open it**.
- Discard all consumables used for the analysis.
- Using an alcohol-based solution and an absorbent cloth, clean the work area, tools (pipettes, stands, etc.) and equipment (LoopiX®, PurifiX®, etc.)
- Perform a 30-minute UV sterilisation cycle for equipment featuring this function.

SWA_12 Surface swabbing / PK / Centrifugation / PurifiX®10



MIL_09 – Enriched medium / PK / Centrifugation / PFS+ manual purification

Sampling

- Prepare the sample for analysis in accordance with applicable standards or the company's internal procedures (enrichment or revitalisation if necessary), giving preference to the use of filter bags to retain solid particles.
- Homogenise the sample, then transfer **200 µL** into the **L1** lysis tube.

Lysis

- Using a pipette fitted with a new tip, transfer **25 µL** of **liquid PK** into tube **L1** and mix thoroughly by pipetting back and forth.
- Close tube **L1** and place it in the LoopiX®.
- Using the control interface, start a '**PK and amplification**' analysis. The indicator light flashes red throughout the lysis process.
- At the end of lysis, the indicator light flashes blue. Remove tube **L1** from the LoopiX®.
- Using a pipette fitted with a tip or a 6 mL transfer pipette, aspirate the entire contents of tube **L1**.

Centrifugation

- Whilst lysis is taking place, prepare the separation column:
 - Take a separation column and detach it from the **C1** collection tube.
 - Remove the cap from the column.
 - Reinsert the column into the collection tube.
- Transfer the entire sample from tube **L1** into the separation column.
- Close the column and place it in the centrifuge. Balance the centrifuge.
- Start centrifugation at **2500 xg** (maximum 6000 xg) for **2 minutes**.

NB: The time/speed combination may be set specifically according to the centrifuge model; please specify the relevant data below:

Speed	Duration

- After centrifugation, remove the separation column from its collection tube **C1**. Discard the column.
- Using a pipette fitted with a new tip, or a new 6 mL bulb pipette, collect all of the filtrate from tube **C1**.

If a pellet has formed at the bottom of tube **C1**, ensure that only the supernatant is collected.

Purification with PFS+ kit

- Transfer the lysate (liquid remaining after the lysis step) to tube **B2** using a 6 mL pipette or a pipette fitted with a tip.
- Homogenise by aspirating and dispensing 15 times, ensuring the beads are evenly distributed without clumping.
- Wait 1 minute, then place tube **B2** on the magnetic stand to capture the beads.

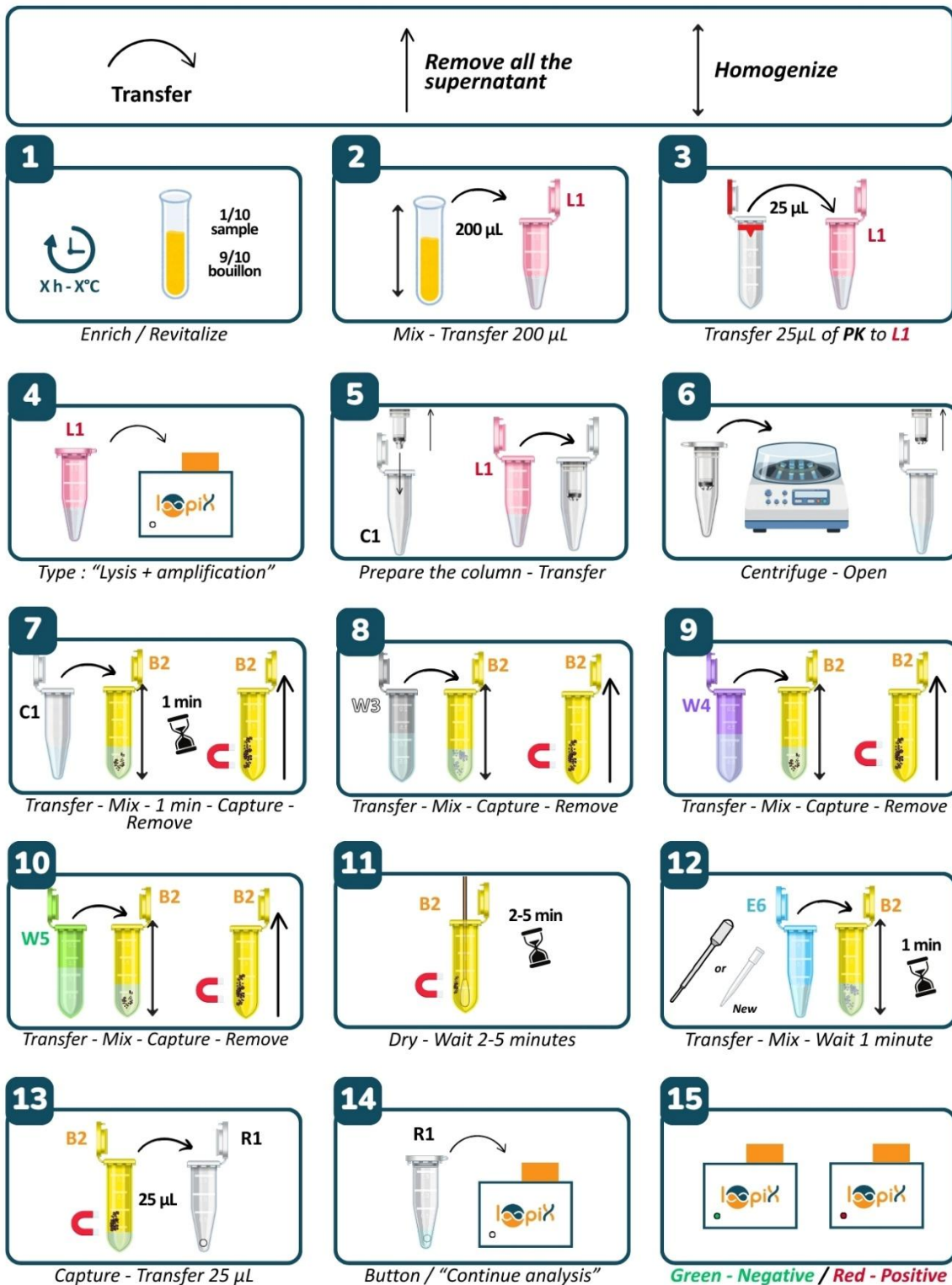
- Discard the supernatant without touching the beads, leaving **B2** on the stand.
- Add buffer **W3** and homogenise again.
- Place tube **B2** on the magnetic stand to capture the beads.
- Discard the supernatant without touching the beads; tube **B2** remains on the stand.
- Add buffer **W4** and repeat the steps for homogenisation, bead capture and removal of the supernatant.
- Add buffer **W5** and repeat the same steps of homogenisation, bead capture and supernatant removal.
- Dry the bottom of the tube with a Lemoine swab or a pipette fitted with a new tip (nothing should remain at the bottom of the tube), then discard the swab or tip.
- Leave to air dry for 5 minutes with tube **B2** open.
- Using a 3.5 mL pipette or a pipette fitted with a new tip, add buffer **E6** and homogenise. Wait 1 minute.
- Place tube **B2** on the magnetic stand to capture the beads.
- Using a pipette fitted with a new tip, withdraw **25 µL** from tube **B2** without touching the beads.

Analysis

- Transfer the **25 µL** to reaction tube **R1**.
- Seal tube **R1** by pressing down with your finger until you hear a 'click' indicating that it is securely closed, then place it in the LoopiX®.
- Start the analysis by pressing the button on the front of the LoopiX® or via the control interface.
- At the end of the analysis, read the result directly from the status LED and on the control interface:
 - **Red LED:** positive sample
 - **Green LED:** negative sample

End of analysis

- Discard the **R1** reaction tube and **NEVER open it**.
- Discard all consumables used for the analysis.
- Using an alcohol-based solution and an absorbent cloth, clean the work area, tools (pipettes, stands, etc.) and equipment (LoopiX®, PurifiX®, etc.)
- Perform a 30-minute UV sterilisation cycle for equipment featuring this function.



MIL_10 - Enriched medium / PK / LoopiX® lysis / Centrifugation / PurifiX®8 purification

Sampling

- Prepare the sample for analysis in accordance with applicable standards or the company's internal procedures (enrichment or revitalisation if necessary), giving preference to the use of filter bags to retain solid particles.
- Homogenise the sample, then transfer **200 µL** into the **L1** lysis tube.

Lysis

- Using a pipette fitted with a new tip, transfer **25 µL** of **liquid PK** into tube **L1** and mix thoroughly by pipetting back and forth.
- Close tube **L1** and place it in the LoopiX®.
- Using the control interface, start a '**PK and amplification**' analysis. The indicator light flashes red throughout the lysis process.
- At the end of lysis, the indicator light flashes blue. Remove tube **L1** from the LoopiX®.
- Using a pipette fitted with a tip or a 6 mL transfer pipette, aspirate the entire contents of tube **L1**.

Centrifugation

- Whilst lysis is taking place, prepare the separation column:
 - Take a separation column and detach it from the **C1** collection tube.
 - Remove the cap from the column.
 - Reinsert the column into the collection tube.
- Transfer the entire sample from tube **L1** into the separation column.
- Close the column and place it in the centrifuge. Balance the centrifuge.
- Start centrifugation at **2500 xg** (maximum 6000 xg) for **2 minutes**.

NB: The time/speed combination may be set specifically according to the centrifuge model; please specify the relevant data below:

Speed	Duration

- After centrifugation, remove the separation column from its collection tube **C1**. Discard the column.
- Using a pipette fitted with a new tip, or a new 6 mL bulb pipette, collect all of the filtrate from tube **C1**.

If a pellet has formed at the bottom of tube **C1, ensure that only the supernatant is collected.**

PurifiX® 8 purification

- Transfer the lysate (liquid remaining after the lysis step) to **well 1** of the PurifiX® 8 purification strip using a 6 mL pipette or a pipette fitted with a tip.
- Place the purification strip onto the holder in the PurifiX® 8.
- Insert the comb(s) into the device, ensuring they are pushed all the way in.

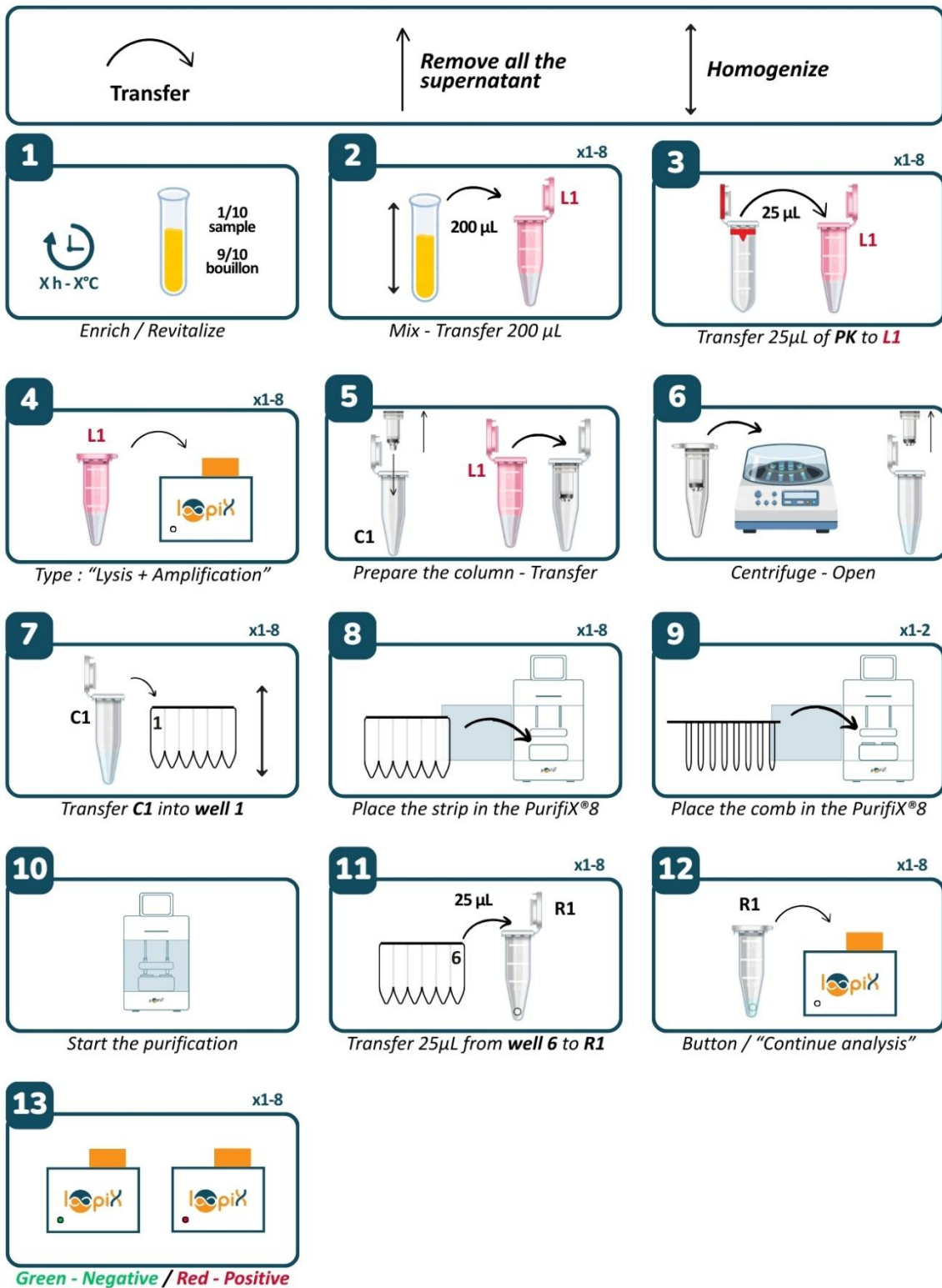
- Close the instrument lid and start the run using the control interface.
- At the end of the purification, discard the comb(s) and remove the purification strip.
- Using a pipette fitted with a **new tip**, aspirate **25 µL** from **well 6** of the strip and proceed with the analysis.

Analysis

- Transfer the **25 µL** to reaction tube **R1**.
- Seal tube **R1** by pressing down with your finger until you hear a 'click' indicating that it is securely closed, then place it in the LoopiX®.
- Start the analysis by pressing the button on the front of the LoopiX® or via the control interface.
- At the end of the analysis, read the result directly from the status LED and on the control interface:
 - **Red LED:** positive sample
 - **Green LED:** negative sample

End of analysis

- Discard the **R1** reaction tube and **NEVER open it**.
- Discard all consumables used for the analysis.
- Using an alcohol-based solution and an absorbent cloth, clean the work area, tools (pipettes, stands, etc.) and equipment (LoopiX®, PurifiX®, etc.)
- Perform a 30-minute UV sterilisation cycle for equipment featuring this function.



MIL_11 - Enriched medium / PK / Heating block / Centrifugation / PurifiX®8 purification

Sampling

- Prepare the sample for analysis in accordance with applicable standards or the company's internal procedures (enrichment or revitalisation if necessary), giving preference to the use of filter bags to retain solid particles.
- Homogenise the sample, then transfer **200 µL** into the **L1** lysis tube.

Lysis

- Using a pipette fitted with a new tip, transfer **25 µL** of **liquid PK** into tube **L1** and mix by pipetting back and forth.
- Seal tube **L1** and place it in the heating block preheated to **65°C**.
- Set a timer for **10 minutes**.
- Once lysis is complete, remove tube **L1** from the heating block and wait 2 minutes for it to cool.
- Using a pipette fitted with a tip or a 6 mL transfer pipette, aspirate the entire contents of tube **L1**.

Centrifugation

- Whilst lysis is taking place, prepare the separation column:
 - Take a separation column and detach it from the **C1** collection tube.
 - Remove the cap from the column.
 - Reinsert the column into the collection tube.
- Transfer the entire sample from tube **L1** into the separation column.
- Close the column and place it in the centrifuge. Balance the centrifuge.
- Start centrifugation at **2500 xg** (maximum 6000 xg) for **2 minutes**.

NB: The time/speed combination may be set specifically according to the centrifuge model; please specify the relevant data below:

Speed	Duration

- After centrifugation, remove the separation column from its collection tube **C1**. Discard the column.
- Using a pipette fitted with a new tip, or a new 6 mL bulb pipette, collect all of the filtrate from tube **C1**.

If a pellet has formed at the bottom of tube **C1**, ensure that only the supernatant is collected.

PurifiX® 8 purification

- Transfer the lysate (liquid remaining after the lysis step) to **well 1** of the PurifiX® 8 purification strip using a 6 mL pipette or a pipette fitted with a tip.
- Place the purification strip onto the holder in the PurifiX® 8.
- Insert the comb(s) into the device, ensuring they are pushed all the way in.
- Close the instrument lid and start the run using the control interface.

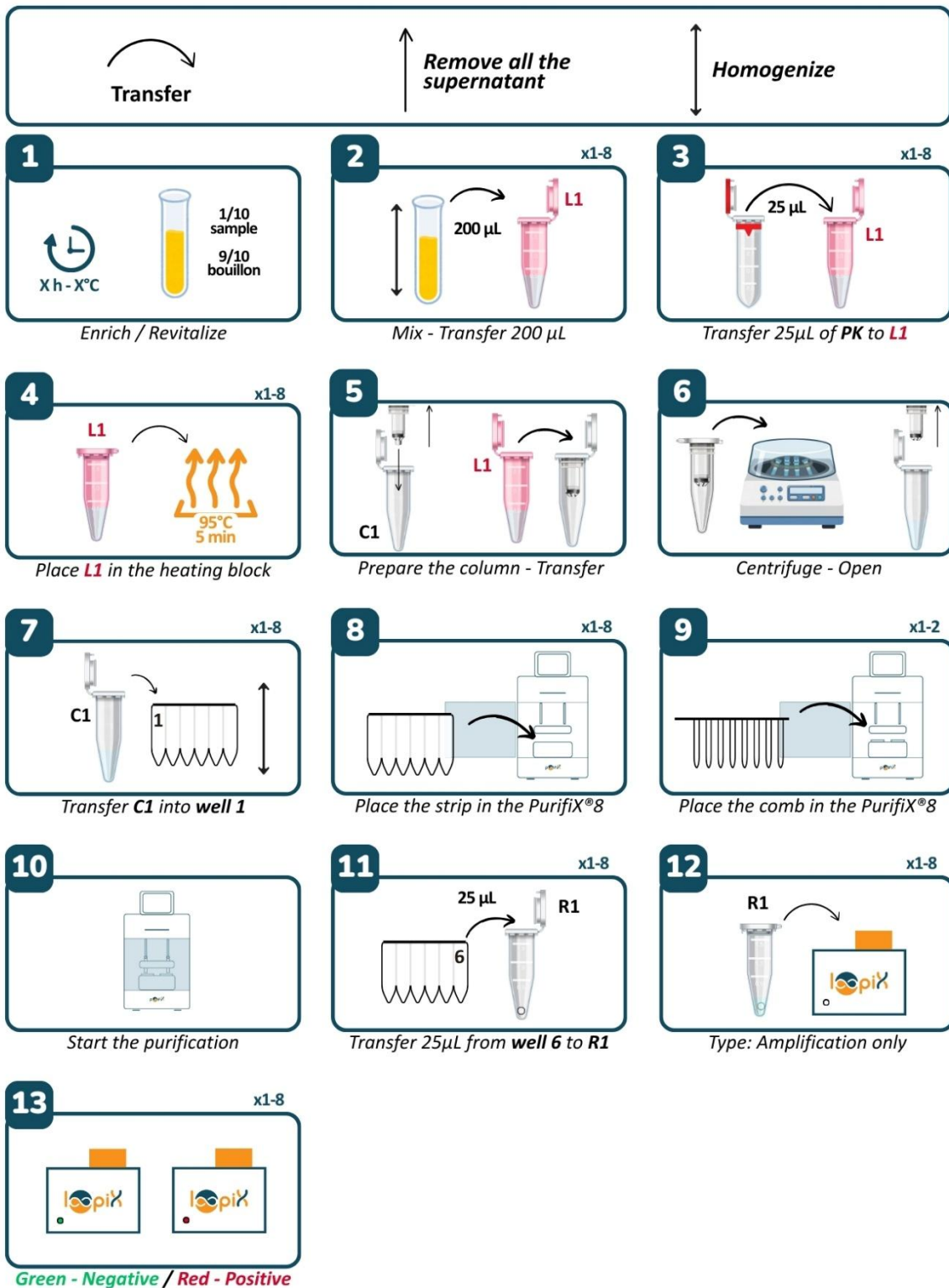
- At the end of the purification, discard the comb(s) and remove the purification strip.
- Using a pipette fitted with a **new tip**, aspirate **25 µL** from **well 6** of the strip and proceed with the analysis.

Analysis

- Transfer the **25 µL** to reaction tube **R1**.
- Seal tube **R1** by pressing down with your finger until you hear a 'click' indicating that it is securely closed, then place it in the LoopiX®.
- Start the analysis by pressing the button on the front of the LoopiX® or via the control interface.
- At the end of the analysis, read the result directly from the status LED and on the control interface:
 - **Red LED:** positive sample
 - **Green LED:** negative sample

End of analysis

- Discard the **R1** reaction tube and **NEVER open it**.
- Discard all consumables used for the analysis.
- Using an alcohol-based solution and an absorbent cloth, clean the work area, tools (pipettes, stands, etc.) and equipment (LoopiX®, PurifiX®, etc.)
- Perform a 30-minute UV sterilisation cycle for equipment featuring this function.



MIL_12 - Enriched medium / PK / Centrifugation / PurifiX®10 purification

Sampling

- Prepare the sample for analysis in accordance with applicable standards or the company's internal procedures (enrichment or revitalisation if necessary), giving preference to the use of filter bags to retain solid particles.
- Homogenise the sample, then transfer **200 µL** into the **L1** lysis tube.

Lysis

- Using a pipette fitted with a new tip, transfer **25 µL** of **liquid PK** into tube **L1** and mix by pipetting back and forth.
- Seal tube **L1** and place it in the heating block preheated to **65°C**.
- Set a timer for **10 minutes**.
- Once lysis is complete, remove tube **L1** from the heating block and wait 2 minutes for it to cool.
- Using a pipette fitted with a tip or a 6 mL transfer pipette, aspirate the entire contents of tube **L1**.

Centrifugation

- Whilst lysis is taking place, prepare the separation column:
 - Take a separation column and detach it from the **C1** collection tube.
 - Remove the cap from the column.
 - Reinsert the column into the collection tube.
- Transfer the entire sample from tube **L1** into the separation column.
- Close the column and place it in the centrifuge. Balance the centrifuge.
- Start centrifugation at **2500 xg** (maximum 6000 xg) for **2 minutes**.

NB: The time/speed combination may be set specifically according to the centrifuge model; please specify the relevant data below:

Speed	Duration

- After centrifugation, remove the separation column from its collection tube **C1**. Discard the column.
- Using a pipette fitted with a new tip, or a new 6 mL bulb pipette, collect all of the filtrate from tube **C1**.

If a pellet has formed at the bottom of tube **C1, ensure that only the supernatant is collected.**

PurifiX® 10 purification

- Transfer the lysate (liquid remaining after the lysis phase) to **well 1** of the PurifiX® 10 purification strip using a 6 mL pipette or a pipette fitted with a tip.
- Place the purification strip into the PurifiX® 10.
- Place the comb into the rail, ensuring it is pushed all the way in.
- Close the instrument lid and start the run using the control interface.

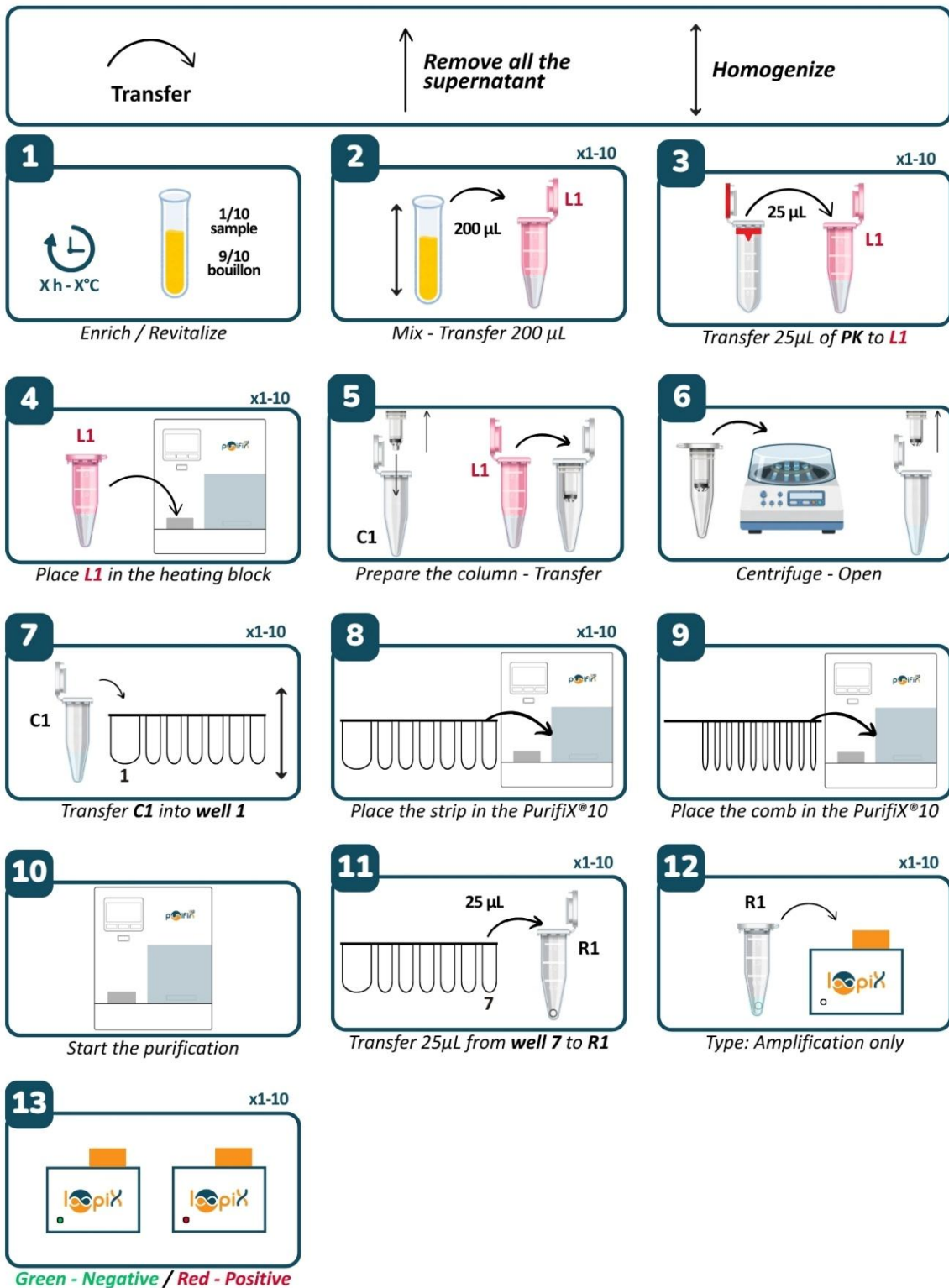
- Once purification is complete, discard the comb and remove the purification strip.
- Using a pipette fitted with a new tip, withdraw **25 µL** from **well 7** of the strip and proceed with the analysis.

Analysis

- Transfer the **25 µL** to reaction tube **R1**.
- Seal tube **R1** by pressing down with your finger until you hear a 'click' indicating that it is securely closed, then place it in the LoopiX®.
- Start the analysis by pressing the button on the front of the LoopiX® or via the control interface.
- At the end of the analysis, read the result directly from the status LED and on the control interface:
 - **Red LED:** positive sample
 - **Green LED:** negative sample

End of analysis

- Discard the **R1** reaction tube and **NEVER open it**.
- Discard all consumables used for the analysis.
- Using an alcohol-based solution and an absorbent cloth, clean the work area, tools (pipettes, stands, etc.) and equipment (LoopiX®, PurifiX®, etc.)
- Perform a 30-minute UV sterilisation cycle for equipment featuring this function.



COL_09 – Colony isolated from agar plate / PK / Centrifugation / PFS+ manual purification

Sampling

- Using an inoculation loop, take a colony and resuspend it in lysis tube **L1**.

Lysis

- Using a pipette fitted with a new tip, transfer **25 µL** of **liquid PK** into tube **L1** and mix thoroughly by pipetting back and forth.
- Close tube **L1** and place it in the LoopiX®.
- Using the control interface, start a '**PK and amplification**' analysis. The indicator light flashes red throughout the lysis process.
- At the end of lysis, the indicator light flashes blue. Remove tube **L1** from the LoopiX®.
- Using a pipette fitted with a tip or a 6 mL transfer pipette, aspirate the entire contents of tube **L1**.

Centrifugation

- Whilst lysis is taking place, prepare the separation column:
 - Take a separation column and detach it from the **C1** collection tube.
 - Remove the cap from the column.
 - Reinsert the column into the collection tube.
- Transfer the entire sample from tube **L1** into the separation column.
- Close the column and place it in the centrifuge. Balance the centrifuge.
- Start centrifugation at **2500 xg** (maximum 6000 xg) for **2 minutes**.

NB: The time/speed combination may be set specifically according to the centrifuge model; please specify the relevant data below:

Speed	Duration

- After centrifugation, remove the separation column from its collection tube **C1**. Discard the column.
- Using a pipette fitted with a new tip, or a new 6 mL bulb pipette, collect all of the filtrate from tube **C1**.

If a pellet has formed at the bottom of tube **C1**, ensure that only the supernatant is collected.

Purification with PFS+ kit

- Transfer the lysate (liquid remaining after the lysis step) to tube **B2** using a 6 mL pipette or a pipette fitted with a tip.
- Homogenise by aspirating and dispensing 15 times, ensuring the beads are evenly distributed without clumping.
- Wait 1 minute, then place tube **B2** on the magnetic stand to capture the beads.
- Discard the supernatant without touching the beads, leaving **B2** on the stand.
- Add buffer **W3** and homogenise again.

- Place tube **B2** on the magnetic stand to capture the beads.
- Discard the supernatant without touching the beads; tube **B2** remains on the stand.
- Add buffer **W4** and repeat the steps for homogenisation, bead capture and removal of the supernatant.
- Add buffer **W5** and repeat the same steps of homogenisation, bead capture and supernatant removal.
- Dry the bottom of the tube with a Lemoine swab or a pipette fitted with a **new tip** (nothing should remain at the bottom of the tube), then discard the swab or tip.
- Leave to air dry for 5 minutes with tube **B2** open.
- Using a 3.5 mL pipette or a pipette fitted with a new tip, add buffer **E6** and homogenise. Wait 1 minute.
- Place tube **B2** on the magnetic stand to capture the beads.
- Using a pipette fitted with a **new tip**, withdraw **25 µL** from tube **B2** without touching the beads.

Analysis

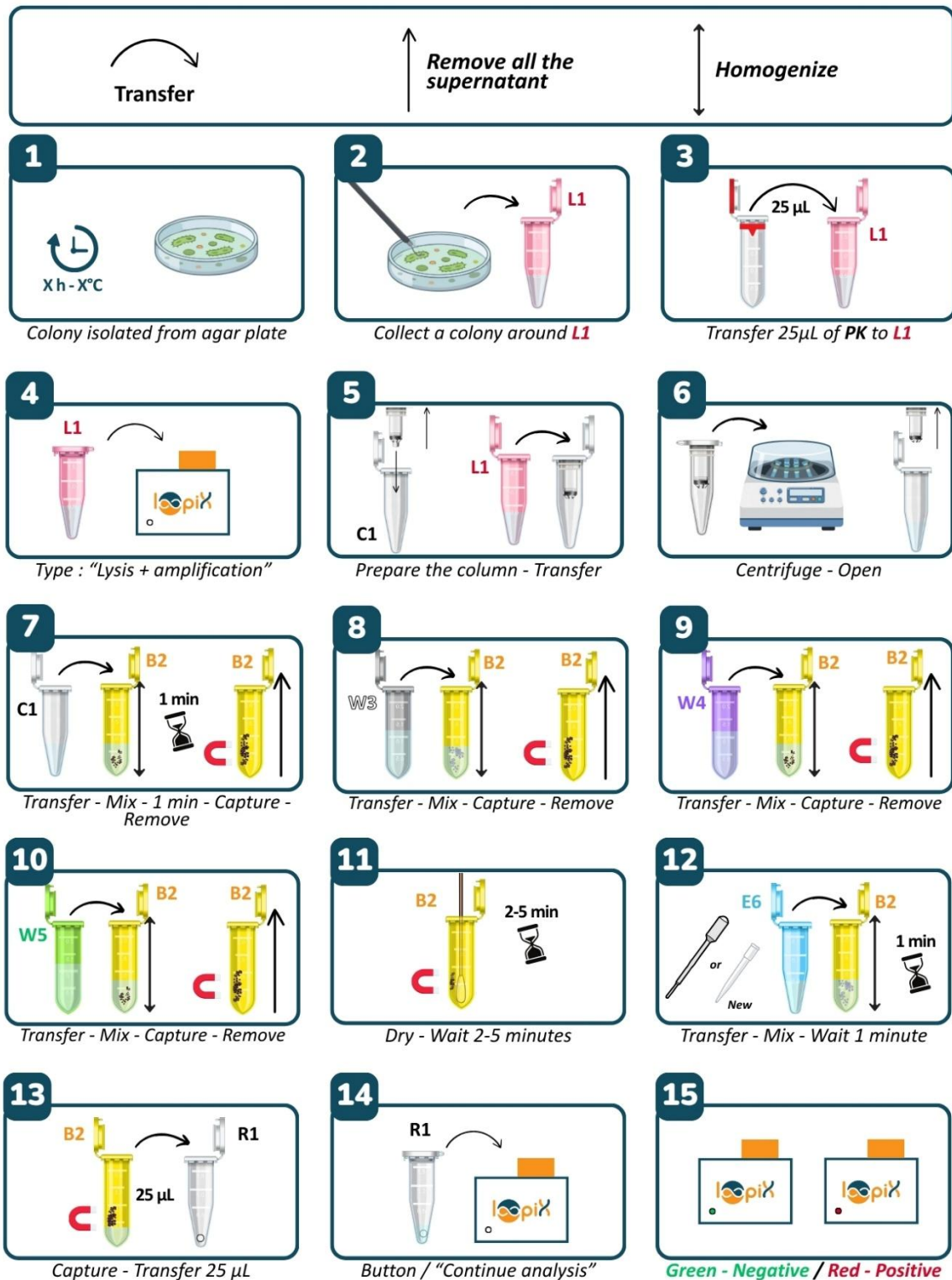
- Transfer the **25 µL** to reaction tube **R1**.
- Seal tube **R1** by pressing down with your finger until you hear a 'click' indicating that it is securely closed, then place it in the LoopiX®.
- Start the analysis by pressing the button on the front of the LoopiX® or via the control interface.
- At the end of the analysis, read the result directly from the status LED and on the control interface:
 - **Red LED:** positive sample
 - **Green LED:** negative sample

End of analysis

- Discard the **R1** reaction tube and **NEVER open it**.
- Discard all consumables used for the analysis.
- Using an alcohol-based solution and an absorbent cloth, clean the work area, tools (pipettes, stands, etc.) and equipment (LoopiX®, PurifiX®, etc.)
- Perform a 30-minute UV sterilisation cycle for equipment featuring this function.

COL_09

Colony isolated from agar plate / PK Lysis LoopiX® / Centrifugation
/ PFS+ manual purification



COL_10 – Colony isolated from agar plate / PK / LoopiX® lysis / Centrifugation / PurifiX®8 purification

Sampling

- Using an inoculation loop, take a colony and resuspend it in lysis tube **L1**.

Lysis

- Using a pipette fitted with a new tip, transfer **25 µL** of **liquid PK** into tube **L1** and mix thoroughly by pipetting back and forth.
- Close tube **L1** and place it in the LoopiX®.
- Using the control interface, start a '**PK and amplification**' analysis. The indicator light flashes red throughout the lysis process.
- At the end of lysis, the indicator light flashes blue. Remove tube **L1** from the LoopiX®.
- Using a pipette fitted with a tip or a 6 mL transfer pipette, aspirate the entire contents of tube **L1**.

Centrifugation

- Whilst lysis is taking place, prepare the separation column:
 - Take a separation column and detach it from the **C1** collection tube.
 - Remove the cap from the column.
 - Reinsert the column into the collection tube.
- Transfer the entire sample from tube **L1** into the separation column.
- Close the column and place it in the centrifuge. Balance the centrifuge.
- Start centrifugation at **2500 xg** (maximum 6000 xg) for **2 minutes**.

NB: The time/speed combination may be set specifically according to the centrifuge model; please specify the relevant data below:

Speed	Duration

- After centrifugation, remove the separation column from its collection tube **C1**. Discard the column.
- Using a pipette fitted with a new tip, or a new 6 mL bulb pipette, collect all of the filtrate from tube **C1**.

If a pellet has formed at the bottom of tube **C1**, ensure that only the supernatant is collected.

PurifiX® 8 purification

- Transfer the lysate (liquid remaining after the lysis step) to **well 1** of the PurifiX® 8 purification strip using a 6 mL pipette or a pipette fitted with a tip.
- Place the purification strip onto the holder in the PurifiX® 8.
- Insert the comb(s) into the device, ensuring they are pushed all the way in.
- Close the instrument lid and start the run using the control interface.
- At the end of the purification, discard the comb(s) and remove the purification strip.

- Using a pipette fitted with a **new tip**, aspirate **25 µL** from **well 6** of the strip and proceed with the analysis.

Analysis

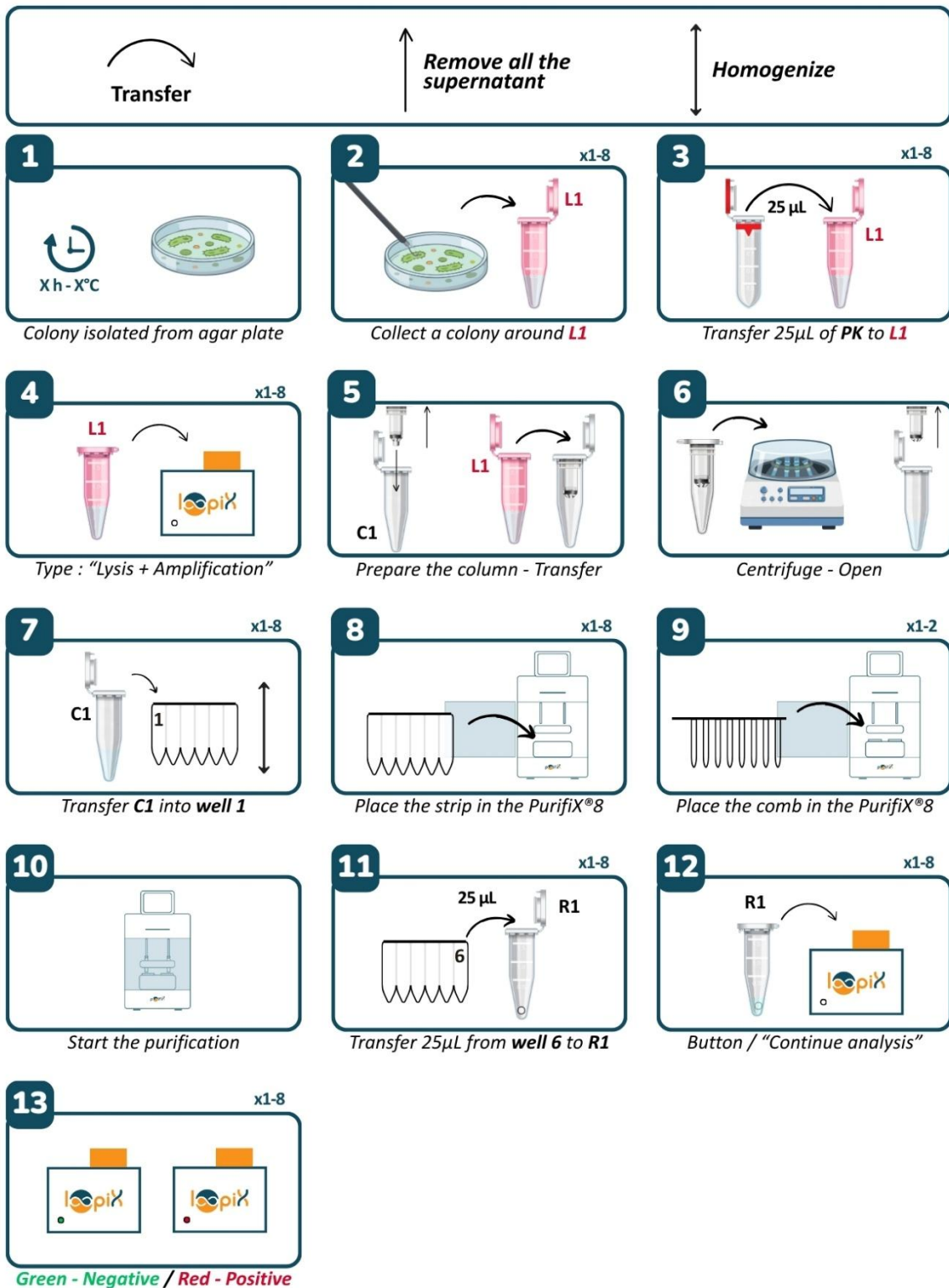
- Transfer the **25 µL** to reaction tube **R1**.
- Seal tube **R1** by pressing down with your finger until you hear a 'click' indicating that it is securely closed, then place it in the LoopiX®.
- Start the analysis by pressing the button on the front of the LoopiX® or via the control interface.
- At the end of the analysis, read the result directly from the status LED and on the control interface:
 - **Red LED:** positive sample
 - **Green LED:** negative sample

End of analysis

- Discard the **R1** reaction tube and **NEVER open it**.
- Discard all consumables used for the analysis.
- Using an alcohol-based solution and an absorbent cloth, clean the work area, tools (pipettes, stands, etc.) and equipment (LoopiX®, PurifiX®, etc.)
- Perform a 30-minute UV sterilisation cycle for equipment featuring this function.

COL_10

Colony isolated from agar plate / PK Lysis LoopiX® / Centrifugation / PurifiX®8



COL_11 – Colony isolated from agar plate / PK / Heating block / Centrifugation / PurifiX®8 purification

Sampling

- Using an inoculation loop, take a colony and resuspend it in lysis tube **L1**.

Lysis

- Using a pipette fitted with a new tip, transfer **25 µL** of **liquid PK** into tube **L1** and mix by pipetting back and forth.
- Seal tube **L1** and place it in the heating block preheated to **65°C**.
- Set a timer for **10 minutes**.
- Once lysis is complete, remove tube **L1** from the heating block and wait 2 minutes for it to cool.
- Using a pipette fitted with a tip or a 6 mL transfer pipette, aspirate the entire contents of tube **L1**.

Centrifugation

- Whilst lysis is taking place, prepare the separation column:
 - Take a separation column and detach it from the **C1** collection tube.
 - Remove the cap from the column.
 - Reinsert the column into the collection tube.
- Transfer the entire sample from tube **L1** into the separation column.
- Close the column and place it in the centrifuge. Balance the centrifuge.
- Start centrifugation at **2500 xg** (maximum 6000 xg) for **2 minutes**.

NB: The time/speed combination may be set specifically according to the centrifuge model; please specify the relevant data below:

Speed	Duration

- After centrifugation, remove the separation column from its collection tube **C1**. Discard the column.
- Using a pipette fitted with a new tip, or a new 6 mL bulb pipette, collect all of the filtrate from tube **C1**.

If a pellet has formed at the bottom of tube **C1**, ensure that only the supernatant is collected.

PurifiX® 8 purification

- Transfer the lysate (liquid remaining after the lysis step) to **well 1** of the PurifiX® 8 purification strip using a 6 mL pipette or a pipette fitted with a tip.
- Place the purification strip onto the holder in the PurifiX® 8.
- Insert the comb(s) into the device, ensuring they are pushed all the way in.
- Close the instrument lid and start the run using the control interface.
- At the end of the purification, discard the comb(s) and remove the purification strip.

- Using a pipette fitted with a **new tip**, aspirate **25 µL** from **well 6** of the strip and proceed with the analysis.

Analysis

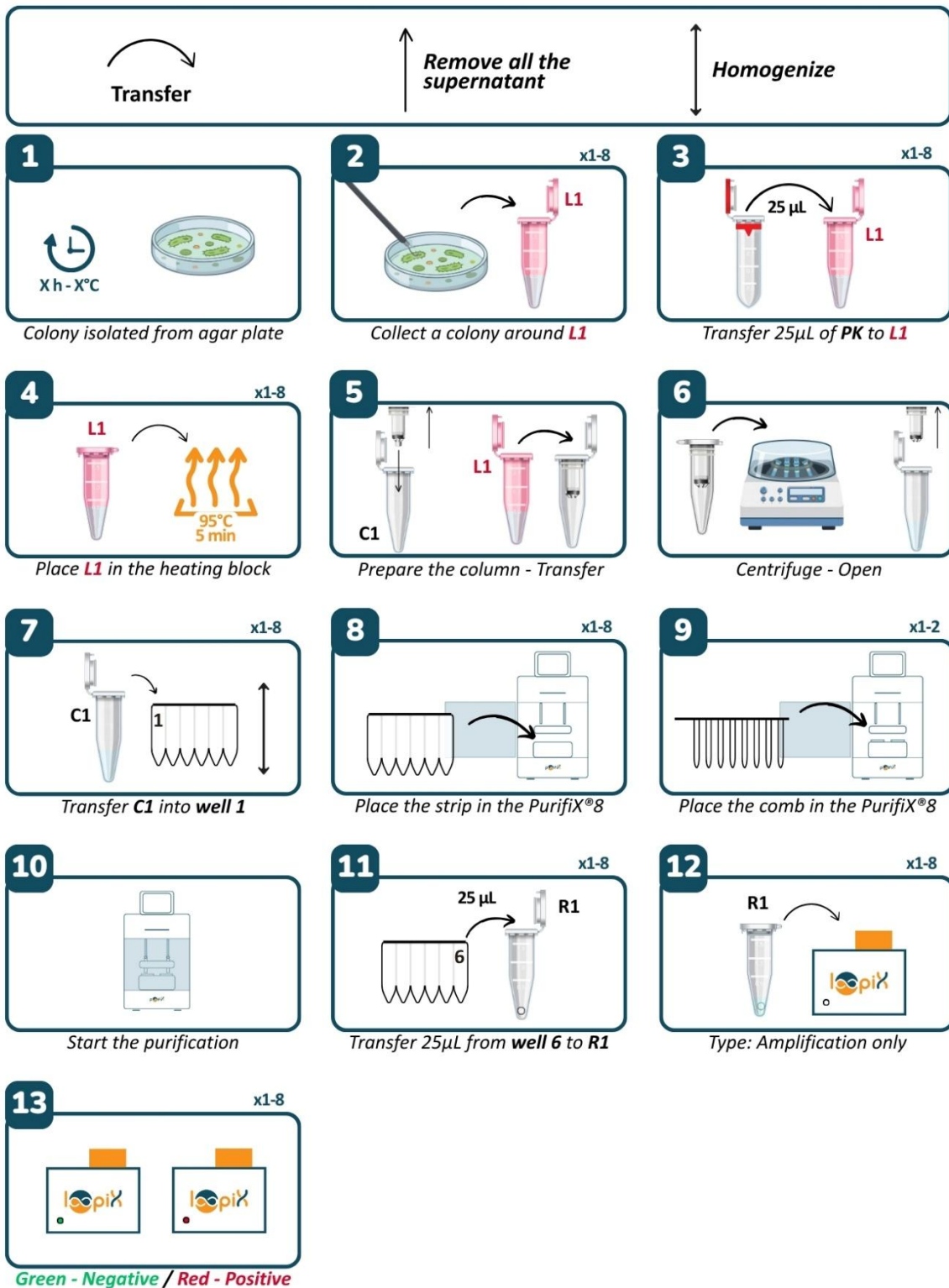
- Transfer the **25 µL** to reaction tube **R1**.
- Seal tube **R1** by pressing down with your finger until you hear a 'click' indicating that it is securely closed, then place it in the LoopiX®.
- Start the analysis by pressing the button on the front of the LoopiX® or via the control interface.
- At the end of the analysis, read the result directly from the status LED and on the control interface:
 - **Red LED:** positive sample
 - **Green LED:** negative sample

End of analysis

- Discard the **R1** reaction tube and **NEVER open it**.
- Discard all consumables used for the analysis.
- Using an alcohol-based solution and an absorbent cloth, clean the work area, tools (pipettes, stands, etc.) and equipment (LoopiX®, PurifiX®, etc.)
- Perform a 30-minute UV sterilisation cycle for equipment featuring this function.

COL_11

Colony isolated from agar plate / PK Heating block / Centrifugation / PurifiX®8



COL_12 – Colony isolated from agar plate / PK / Centrifugation / PurifiX®10 purification

Sampling

- Using an inoculation loop, take a colony and resuspend it in lysis tube **L1**.

Lysis

- Using a pipette fitted with a new tip, transfer **25 µL** of **liquid PK** into tube **L1** and mix by pipetting back and forth.
- Seal tube **L1** and place it in the heating block preheated to **65°C**.
- Set a timer for **10 minutes**.
- Once lysis is complete, remove tube **L1** from the heating block and wait 2 minutes for it to cool.
- Using a pipette fitted with a tip or a 6 mL transfer pipette, aspirate the entire contents of tube **L1**.

Centrifugation

- Whilst lysis is taking place, prepare the separation column:
 - Take a separation column and detach it from the **C1** collection tube.
 - Remove the cap from the column.
 - Reinsert the column into the collection tube.
- Transfer the entire sample from tube **L1** into the separation column.
- Close the column and place it in the centrifuge. Balance the centrifuge.
- Start centrifugation at **2500 xg** (maximum 6000 xg) for **2 minutes**.

NB: The time/speed combination may be set specifically according to the centrifuge model; please specify the relevant data below:

Speed	Duration

- After centrifugation, remove the separation column from its collection tube **C1**. Discard the column.
- Using a pipette fitted with a new tip, or a new 6 mL bulb pipette, collect all of the filtrate from tube **C1**.

If a pellet has formed at the bottom of tube **C1**, ensure that only the supernatant is collected.

PurifiX® 10 purification

- Transfer the lysate (liquid remaining after the lysis phase) to **well 1** of the PurifiX® 10 purification strip using a 6 mL pipette or a pipette fitted with a tip.
- Place the purification strip into the PurifiX® 10.
- Place the comb into the rail, ensuring it is pushed all the way in.
- Close the instrument lid and start the run using the control interface.
- Once purification is complete, discard the comb and remove the purification strip.
- Using a pipette fitted with a new tip, withdraw **25 µL** from **well 7** of the strip and proceed with the analysis.

Analysis

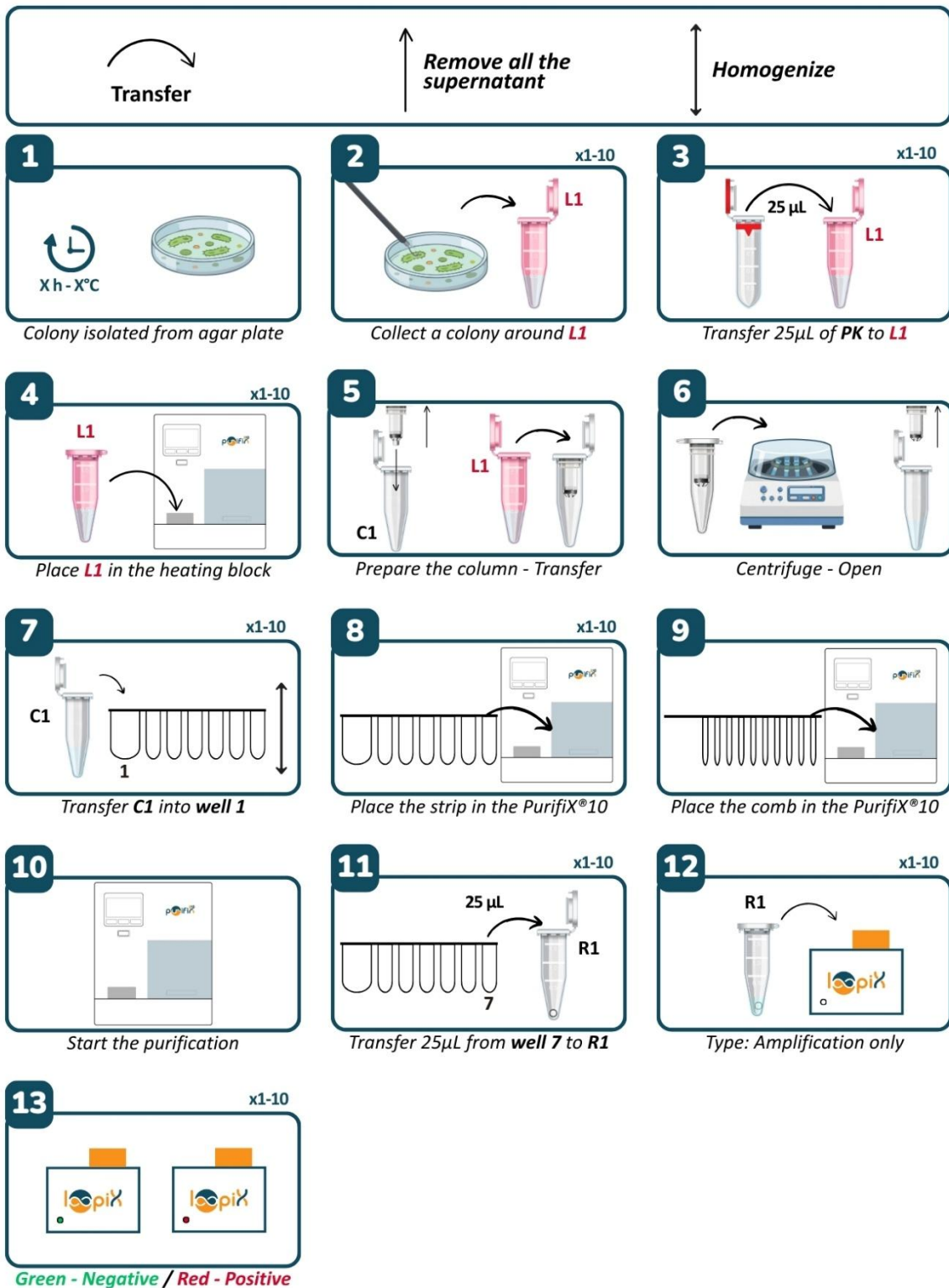
- Transfer the **25 µL** to reaction tube **R1**.
- Seal tube **R1** by pressing down with your finger until you hear a 'click' indicating that it is securely closed, then place it in the LoopiX®.
- Start the analysis by pressing the button on the front of the LoopiX® or via the control interface.
- At the end of the analysis, read the result directly from the status LED and on the control interface:
 - **Red LED:** positive sample
 - **Green LED:** negative sample

End of analysis

- Discard the **R1** reaction tube and **NEVER open it**.
- Discard all consumables used for the analysis.
- Using an alcohol-based solution and an absorbent cloth, clean the work area, tools (pipettes, stands, etc.) and equipment (LoopiX®, PurifiX®, etc.)
- Perform a 30-minute UV sterilisation cycle for equipment featuring this function.

COL_12

Colony isolated from agar plate / PK / Centrifugation / PurifiX®10



NEG_01 – Negative control

Preparation

- Using a pipette fitted with a tip, withdraw **25 µL** from tube **S1**.

Analysis

- Transfer the **25 µL** to reaction tube **R1**.
- Seal tube **R1** by pressing down with your finger until you hear a 'click' indicating that it is securely closed, then place it in the LoopiX®.
- Start the analysis by pressing the button on the front of the LoopiX® or via the control interface.
- At the end of the analysis, read the result directly from the status LED and on the control interface:
 - Red LED:** positive sample
 - Green LED:** negative sample

End of analysis

- Discard the **R1** reaction tube and **NEVER open it**.
- Discard all consumables used for the analysis.
- Using an alcohol-based solution and an absorbent cloth, clean the work area, tools (pipettes, stands, etc.) and equipment (LoopiX®, PurifiX®, etc.)
- Perform a 30-minute UV sterilisation cycle for equipment featuring this function.

POS_01 – Positive control

Material

Warning: You must use the specific positive control from the LoopDeetect kit being tested:

Positive control	Kit content	Related LoopDeetect kit
LoopDeetect <i>Salmonella spp.</i> +	- 1 positive control (yellow tube / red bag) - 1 sample tube (S1)	LoopDeetect <i>Salmonella spp.</i>
LoopDeetect <i>Listeria</i> +	- 1 positive control (yellow tube / red bag) - 1 sample tube (S1)	LoopDeetect <i>Listeria monocytogenes</i>
		LoopDeetect <i>Listeria spp.</i>
LoopDeetect <i>Escherichia coli</i> +	- 1 positive control (yellow tube / red bag) - 1 sample tube (S1)	LoopDeetect <i>Escherichia coli</i>
LoopDeetect <i>Enterococcus faecalis</i> +	- 1 positive control (yellow tube / red bag) - 1 sample tube (S1)	LoopDeetect <i>Enterococcus faecalis</i>

Open the pouch containing the **T+ positive control (yellow tube)**. Check that the tablet is present (blue colouring at the bottom of the tube).

Preparation

- Using a pipette fitted with a tip, withdraw **50 µL** from tube **S1**.

- Transfer the sample to the **T+ tube** of the target, onto the **blue spot**.
- Homogenise by a series of up-and-down pipetting movements.
- Using the pipette, withdraw **25 µL** from the **T+ tube**.

Analysis

- Transfer the **25 µL** to reaction tube **R1**.
- Seal tube **R1** by pressing down with your finger until you hear a 'click' indicating that it is securely closed, then place it in the LoopiX®.
- Start the analysis by pressing the button on the front of the LoopiX® or via the control interface.
- At the end of the analysis, read the result directly from the status LED and on the control interface:
 - **Red LED:** positive sample
 - **Green LED:** negative sample

End of analysis

- Discard the **R1** reaction tube and **NEVER open it**.
- Discard all consumables used for the analysis.
- Using an alcohol-based solution and an absorbent cloth, clean the work area, tools (pipettes, stands, etc.) and equipment (LoopiX®, PurifiX®, etc.)
- Perform a 30-minute UV sterilisation cycle for equipment featuring this function.

INH_01 – Inhibition control

This protocol is used to check the compatibility of the matrix with the LoopDetect method; the principle involves adding the target to the sample after purification in order to confirm that the amplification is correct and that the sample does not contain any inhibitory substances that may not have been removed during the purification step.

This protocol can only be carried out using the purification kits supplied by Loop Dee Science.

Material

Warning : You must use the specific positive control from the LoopDeetect kit being tested:

Positive control	Kit content	Related LoopDeetect lit
LoopDeetect <i>Salmonella</i> spp. +	- 1 positive control (yellow tube / red bag) - 1 sample tube (S1)	LoopDeetect <i>Salmonella</i> spp.
LoopDeetect <i>Listeria</i> +	- 1 positive control (yellow tube / red bag) - 1 sample tube (S1)	LoopDeetect <i>Listeria monocytogenes</i>
		LoopDeetect <i>Listeria</i> spp.
LoopDeetect <i>Escherichia coli</i> +	- 1 positive control (yellow tube / red bag) - 1 sample tube (S1)	LoopDeetect <i>Escherichia coli</i>
LoopDeetect <i>Enterococcus faecalis</i> +	- 1 positive control (yellow tube / red bag) - 1 sample tube (S1)	LoopDeetect <i>Enterococcus faecalis</i>

- Open the sachet containing the **T+ positive control (yellow tube)**. Check that the tablet is present (blue colouring at the bottom of the tube).

Sampling

- Carry out the **sampling** stage in accordance with the protocol for the method used.

Lysis

- Carry out the **lysis** stage in accordance with the protocol for the method used.

Centrifugation

- Carry out the **centrifugation** stage in accordance with the protocol for the method used.
- If the method used does not involve centrifugation, skip this step.

Purification

- Carry out the **purification** stage in accordance with the protocol for the method used.

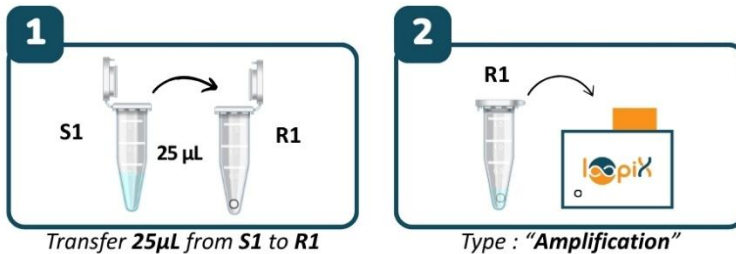
At the end of purification

- Using a pipette fitted with a tip or a transfer pipette, withdraw the entire purified sample.
- Transfer the sample to the target's **T+ tube**, onto the blue disc.
- Homogenise by a series of up-and-down pipetting movements.
- Using a pipette fitted with a tip, withdraw **25 µL** from the target's **T+ tube**.

TEM_01 Controls - Negative / Positive / Inhibition

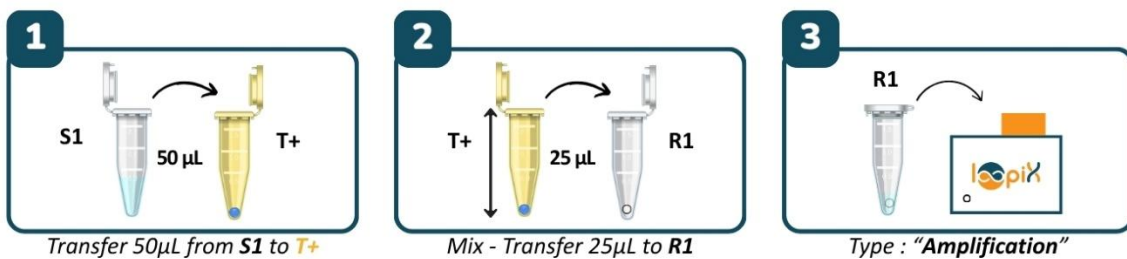


Negative control



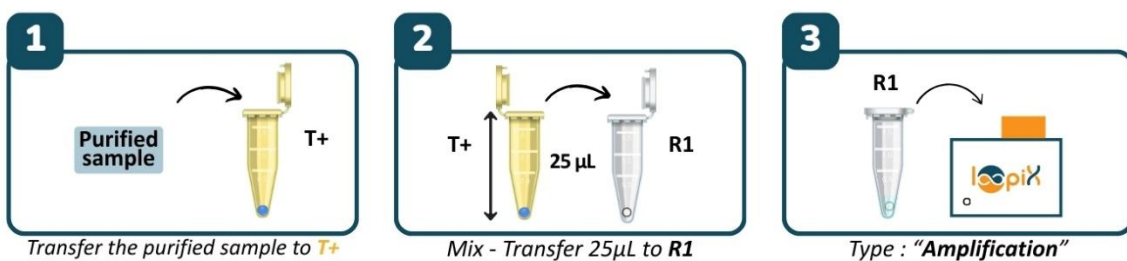
THE RESULT SHOULD BE **GREEN - NEGATIVE**

Positive control



THE RESULT SHOULD BE **RED - POSITIVE**

Inhibition control



THE RESULT SHOULD BE **RED - POSITIVE**

Document history / Version tracking

Version	Date	Comments
1.0	Jan-23	Initial public review
2.0	Juin-23	Changes to operating procedures; spelling corrections
2.1	Nov-23	Changes to product references and packaging – changes to operating procedures.
2.2	Mai-24	Changes to links Addition of LoopiX Cloud
2.3	Juin-24	Change to tube nomenclature: C becomes S Changes to protocols.
3.0	Oct-24	Merger of all instructions for kits in the Food Safety range Changes to protocols Changes to tube nomenclature
4.0	Dec-24	Merger of instructions for Loopdeetect SAL, LMO and LIS kits following the inclusion of MgSO ₄ Changes to protocols Changes to tube nomenclature
5.0	Mars-25	Inclusion of protocols for ECO and EFA analyses Integration of the PFS+ protocol
6.0	Juin-25	Complete revision of protocols Complete revision of flowcharts Corrections
7.0	Nov-25	Update of protocols following improvements to the E. coli kit Update of protocol flowcharts following improvements to the E. coli kit
8.0	Mar-26	Update of protocols Division of the general instruction leaflet into versions according to specific requirements (PK, centrifuge, etc.) Update of protocol names and diagrams. Splitting of protocols and instructions
8.1	June-26	Revision of diagrams 03 and 04 Revision of text PFX10 Purification